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Motor-based microprobe powered by bio-assembled catalase for motion detection of DNA

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ABSTRACT

A motor-based microprobe is proposed using a tubular microengine powered by bio-assembled enzyme as catalyst and exploited for washing-free detection of DNA through motion readout. The microprobe is fabricated by assembling a catalase layer on the inner surface of poly(3,4-ethylenedioxythiophene)/Au (PEDOT/Au) microtube through DNA conjugate, which is responsible for the biocatalytic bubble propulsion. The sensing concept of the microprobe relies on the target-induced release of catalase through the DNA strand-replacement hybridization, which decreases the amount of enzyme assembled on microtube to slow down the movement of the microprobe. Therefore, the motion speed is negatively correlated with the target concentration. At the optimal conditions, the microprobe can conveniently distinguish the concentration of specific DNA in a range of 0.5–10 μM without any washing and separation step. This microprobe can be prepared in batch with good reproducibility and stability, and its motion speed can be conveniently visualized by optical microscope. The proposed motor-based microprobe and its dynamic sensing method provide a novel platform for the development of intelligent microprobe and clinical diagnostic strategy.

1. Introduction

As the carrier of genetic information, DNA is the most important genetic material of living beings. The detection of specific DNA fragments can provide useful information for the diagnosis of many genetic disease and cancers (Drummond et al., 2003; Hay Burgess et al., 2006; Risch and Merikangas, 1996; Wang et al., 1997). Various DNA biosensors along with different signal readouts such as electrochemiluminescence, fluorescence, chemiluminescence, colorimetry and electrochemistry have been reported (Feng et al., 2015; Gao and Li, 2013; Li et al., 2016; Ling et al., 2015; Lou et al., 2015; Shen et al., 2012). Although those biosensors have demonstrated good performance, most of them require sophisticated detection instruments and the complexity manufacturing processes, which greatly limit their applications. Therefore, it is a promising way to design new efficient signal transduction platform for DNA detection by utilizing the unique physical properties, for example, analyzing the motion of micromotors.

Micromotors are self-propelled in liquids by converting different sources of energy into mechanical force and motion (Kagan et al., 2012; Mei et al., 2011; Paxton et al., 2004; Peyer et al., 2013; Sanchez et al., 2015; Solovev et al., 2009), and display a high speed and power, good stability, precise motion control, and self-mixing ability (Gao et al., 2012a, 2012b; Guix et al., 2014; Patra et al., 2013; Wang and Pumera, 2015) in recognizing, transporting, and isolating of a wide range of target biomaterials (Campuzano et al., 2012; Orozco et al., 2013a, 2013b). Recently, powerful synthetic self-propelled nano/micromotors offer considerable promise for developing bioanalytical and biosensing protocols (Bunea et al., 2015; Campuzano et al., 2011; Guix et al., 2014; Wang, 2016; Wang and Pumera, 2015) because the motion sensing platform does not require sophisticated analytical instruments and potentially offers spatial resolution. The idea of motion as a transduction mechanism was firstly introduced by Wang and co-workers (Kagan et al., 2009) and adopted to design the motion-based biosensor for detection of DNA and RNA with a bimetallic nanorod (Wu et al., 2010). To address the limitation of catalytic nanowire motors in high ionic-strength media (Paxton et al., 2006; Sanchez et al., 2015), bubble-propelled tubular micromotors have been designed for the detection of natural toxin and heavy metal ions through their inhibition to catalase (Orozco et al., 2013a, 2013b), cancer biomarkers (Yu et al., 2014) and nucleic acids (Nguyen and Minteer, 2015) due to their efficient bubble-induced propulsion in relevant biological fluids (Gao et al., 2011; Mei et al., 2008). However, the biorecognition layer constructed on the micromotors by the self-assembly of the alkanethiol monolayer and covalent coupling of bioreceptors leads to a significant inhibition to the catalytic layer (Balasubramanian et al., 2011; Campuzano et al.,

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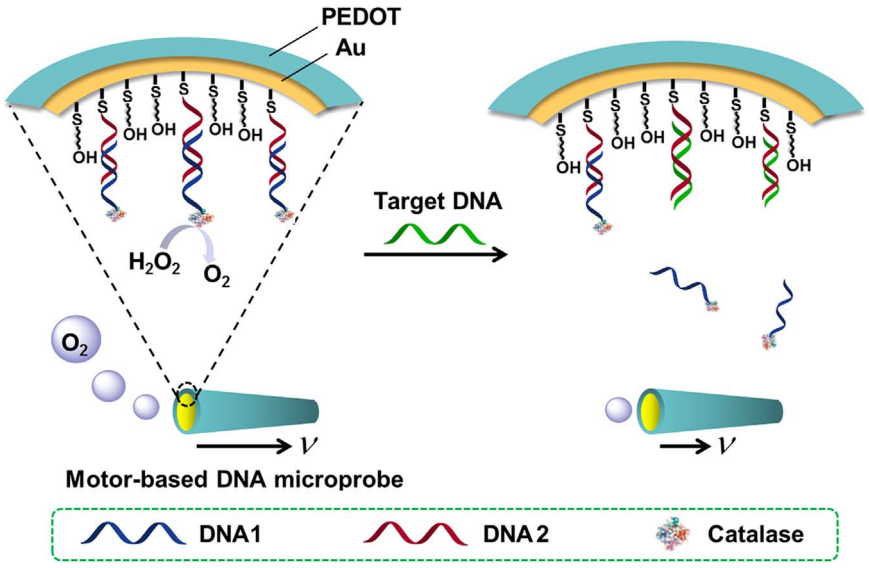

Scheme 1. Schematic representation of motor-based microprobe for DNA detection via motion speed change.

2012). Moreover, the motor-based detection protocol for DNA analysis mostly relies on a sandwich identification assay (Nguyen and Minteer, 2015), which is complex and relatively costly. Here, we use catalase-labeled DNA to present an alternative design of the motor-based DNA microprobe for identification of specific DNA sequence.

Comparing with Pt nanoparticle, catalase as catalyst has higher activity to drive the motion of micromotors through a bubble-induced propulsion mechanism, in which hydrogen peroxide is decomposed to form oxygen gas (Sanchez et al., 2010). In this work, a motor-based microprobe is fabricated by assembly of a catalase layer on the inner surface of poly(3,4-ethylenedioxythiophene)/Au (PEDOT/Au) microtube through DNA conjugate. The motion signal of microprobe is responsible for the biocatalytic bubble propulsion, which can be regulated by the target molecule (Scheme 1). In the presence of target DNA, the DNA1-catalase conjugate is displaced by target DNA, which decreases the amount of enzyme assembled on microtube, and leads to a decrease of the motion speed of microprobe. Thus, a method for convenient and dynamic label-free detection of specific DNA fragments is developed by motion readout. This motor-based microprobe as well as the motion-based detection protocol can be conveniently combined with the developed signal amplification strategies to improve its sensitivity, and extended to detect other analytes such as small molecules and proteins.

2. Materials and methods

2.1. Materials and reagents

3,4-ethylenedioxythiophene (EDOT), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxycinimide (NHS), tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 6-mercapto-1-hexanol (MCH), brilliant blue R and catalase from bovine liver were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Gold plating solution was gifted by Professor Wang Wei at Harbin Institute of Technology (Shenzhen). Sodium cholate hydrate was purchased from Alfa Aesar China Ltd. Sodium dodecyl sulfate (SDS) was obtained from Shanghai Reagent Co. (Shanghai, China). TE buffer (10 mM, pH 7.4) was used to prepare oligonucleotide stock solutions. Phosphate-buffered saline (PBS, 10 mM, pH 5.5) was used as coupling buffer for the immobilization of catalase. Blocking buffer, which was used to block the residual reactive sites on the DNA-modified microtube, was 10 mM PBS (pH 7.4) containing 1 mM MCH. Washing buffer was 10 mM PBS (pH 7.4) spiked with 0.05 wt% SDS. Other reagents were of analytical grade and used as received. Ultrapure water obtained from a Millipore water purification system ($\geq$ 18 MΩ, Milli-Q, Millipore) was used in all experiments. The oligonucleotides with the following sequences were purchased from Shanghai Sangon Biotechnology Co. Ltd. (China) and purified by high-performance liquid chromatography. Their sequences were as follows:

DNA1: 5′-HOOC-TTTTTTTGGTAAAGATGG-3′
DNA2: 5′-HS-TTTTTTCCATCTTTACCAGACAGTGTTA-3′
target DNA: 5′-TAACACTGTCTGGTAAAGATGG-3′
non-complementary DNA: 5′-GATTCGATCTCGACTTCGCATGTACG-3′
single-base mismatch DNA: 5′-TAACAGTGTCTGGTAAAGATGG-3′
three-base mismatch DNA: 5′- TAACAGTGTCTCGTAAACATGG-3′

2.2. Apparatus

Template-assisted electrochemical growth of polymer was carried out with a CHI660B electrochemical workstation (CH Instruments Inc., USA). The morphology of the microprobe was examined with scanning electron microscope (SEM) (Hitachi S-4800, Japan). Zeta potential analysis was performed on Zetasizer (Nano-Z, Malvern, UK). Polyacrylamide gel electrophoresis (PAGE) analysis was performed on an electrophoresis analyser (Bio-Rad, USA) and imaged on Bio-rad ChemDoc XRS (Bio-Rad, USA). The images and videos were captured by Leica DMI 3000B inverted microscope equipped with a Photometrics Evolve 512/SC camera (Roper Scientific, Duluth, GA), and acquired at a frame rate of 10 frames/s using the Leica MM AF 1.5 software.

2.3. Preparation of PEDOT/Au microtubes

The PEDOT/Au microtubes were prepared using a common template-directed electrodeposition protocol (Gao et al., 2012a, 2012b). Briefly, a polycarbonate membrane with 5-μm-diameter micropores (Catalog No 7060-2513, Whatman, U.S.A) was employed as the template. A gold film with a thickness of 75 nm was firstly sputtered on one side of the porous membrane, which was then assembled in a plating cell with an aluminum foil to serve as